0163-4984/(Online) 1559-0720/06/10903–0201 $30.00

Accelerated Article

# Differential Effects of Dietary Selenium (Se) and Folate on Methyl Metabolism in Liver and Colon of Rats*

ERIC O. UTHUS,[1,+] SHARON A. ROSS,[2] AND CINDY D. DAVIS[2]

*[1]USDA-ARS Grand Forks Human Nutrition Research Center, P.O. Box 9034, Grand Forks, ND 58202-9034, and [2]NIH/NCI, Nutritional Sciences Research Group, 6130 Executive Blvd, MSC 7328, Rockville, MD 20892-7328*

Received August 22, 2005; Accepted September 20, 2005

## ABSTRACT

A previous study compared the effects of folate on methyl metabolism in colon and liver of rats fed a selenium-deficient diet (<3 μg Se/kg) to those of rats fed a diet containing supranutritional Se (2 mg selenite/kg). The purpose of this study was to investigate the effects of folate and adequate Se (0.2 mg/kg) on methyl metabolism in colon and liver. Weanling, Fischer-344 rats ($n$=8/diet) were fed diets containing 0 or 0.2 mg selenium (as selenite)/kg and 0 or 2 mg folic acid/kg in a 2 × 2 design. After 70 d, plasma homocysteine was increased ($p$<0.0001) by folate deficiency; this increase was markedly attenuated ($p$<0.0001) in rats fed the selenium-deficient diet compared to those fed 0.2 mg Se/kg. The activity of hepatic glycine *N*-methyltransferase (GNMT), an enzyme involved in the regulation of tissue S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), was increased by folate deficiency ($p$<0.006) and decreased by selenium deprivation ($p$<0.0003). Colon and liver SAH were highest ($p$<0.006) in rats fed deficient folate and adequate selenium. Although folate deficiency decreased liver SAM ($p$<0.001), it had no effect on colon SAM. Global DNA methylation was decreased ($p$<0.04) by selenium deficiency in colon but not liver; folate had no effect. Selenium deficiency did not affect

+ Corresponding author and to whom reprint requests should be addressed: Eric O Uthus, PhD, USDA-ARS Grand Forks Human Nutrition Research Center, P.O. Box 9034, Grand Forks, ND 58202-9034, E-mail:euthus@gfhnrc.ars.usda.gov

* The U.S. Department of Agriculture, Agriculture Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

DNA methyltransferase (Dnmt) activity in liver but tended to decrease ($p<0.06$) the activity of the enzyme in the colon. Dietary folate did not affect liver or colon Dnmt. These results in rats fed adequate selenium are similar to previous results found in rats fed supranutritional selenium. This suggests that selenium deficiency appears to be a more important modifier of methyl metabolism than either adequate or supplemental selenium.

**Index Entries:** Selenium; folate; rat; DNA methylation; one-carbon metabolism.

## INTRODUCTION

Selenium was first associated with cancer risk in the late 1960s. Subsequently, support for that association has come from animal, epidemiologic, and clinical studies. A large body of animal studies has demonstrated the efficacies of both inorganic and organic selenium compounds in inhibiting spontaneous, chemical, and virally induced carcinogenesis *(1).* These studies have shown that the amount of selenium needed to prevent cancer is supranutritional—that is, the amount needed exceeds the nutritional requirement of selenium *(2).* Proposed mechanisms for the cancer-protective properties of selenium include regulation of apoptosis, control of cell cycle progression, modulation of cell signaling molecules, chemopreventive effects of selenoproteins, and modulation of cellular methyl group metabolism, including DNA methylation *(2–7).*

Another nutrient that affects cellular methyl metabolism is folate. Animal studies have shown that folate deficiency can cause DNA hypomethylation prior to the development of tumors *(8).* Epidemiologic studies have observed that diminished folate status is associated with cancer of the cervix, colorectum, lung, esophagus, brain, pancreas, and breast. Among these, epidemiologic support for such a relationship is clearly most compelling for colorectal cancer *(9).* A number of studies have found a reduction in the risk of colorectal adenomas and cancer with long-term dietary supplementation with folic acid. For example, in a large European case-control study, La Vecchia et al. *(10)* found a significantly reduced risk of colorectal cancer when comparing the highest vs. the lowest intakes of folic acid.

There are at least two mechanisms through which selenium and folate may affect DNA methylation and, hence, cancer. First, each nutrient may influence the supply of methyl groups and, therefore, the biochemical pathways of methylation. Second, each may modify utilization of methyl groups by altering DNA methyltransferase (Dnmt) activity.

Previously we determined the interactive effects of dietary selenium and folate on dimethylhydrazine-induced aberrant crypt formation, global DNA methylation, and one-carbon metabolism *(11).* In that study we compared rats fed a selenium-deficient diet (<3 μg Se/kg) to rats fed supranutritional selenium (2 mg Se/kg); folate was fed at either 0 or 2 mg/kg in a

2 × 2 factorial design. We found that selenium deficiency (relative to supranutritional selenium) decreased colonic DNA methylation and the activities of liver DNA methyltransferase and that selenium and folate interacted such that the number of aberrant crypts were the highest and the concentrations of liver S-adenosylmethionine (SAM) were the lowest in rats fed folate-deficient diets supplemented with selenium. However, it was unclear whether the responses to dietary treatment were the result of selenium deprivation or of supranutritional selenium per se. Thus, the purpose of the present study was to investigate the interactive effects of folate and adequate selenium (0.2 mg/kg) to help us determine whether effects of selenium and folate on colon and liver methyl metabolism are the result of selenium deprivation or are possible chemopreventive effects of supranutritional selenium.

## MATERIALS AND METHODS

### *Animals and Diets*

Weanling male Fischer-344 rats ($n$=32) were purchased from Sasco (Omaha, NE). All rats were housed individually in stainless-steel wire-bottomed cages in a room with controlled temperature and light. Rats were provided free access to demineralized water and purified diet. The basal diet was an amino acid–based diet formulated to be low in selenium and folate. The diets contained (on a per kg basis): 186.1 g amino acid mix, 35 g selenium-deficient mineral mix, 10 g folate-deficient vitamin mix, 100 g corn oil, 50 g Alphacel fiber (ICN Pharmaceuticals, Costa Mesa, CA), 2.5 g choline bitartrate, 466.4 g sucrose, and 150 g corn starch. The basal diet was identical to the basal diet that we used in our earlier study on the interaction between selenium (deficient and supranutritional) and folate (deficient and adequate) *(11).* In a 2 × 2 factorial design ($n$ = 8 rats per group), the basal diet was supplemented with 0 or 0.2 mg selenium/kg diet as sodium selenite (by analysis, the basal diet contained <3 μg Se/kg) and with 0 or 2 mg folate/kg. These diets were considered to be either deficient or adequate in selenium and folate because the recommendations for dietary selenium and folate in the AIN-93 diet are 0.15 and 2.0 mg/kg diet, respectively *(12).* Rats consumed the same diets for 10 wk. This study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center, and the rats were maintained in accordance with the guidelines for the care and use of laboratory animals.

### *Sample Collection*

Food was withheld overnight before rats were anesthetized with xylazine (Rompon, Moboay Inc., Shawnee, KS) and ketamine (Ketaset, Aveco Inc., Fort Dodge, IA) and killed by exsanguination. Blood was

collected by cardiac puncture into syringes containing EDTA such that the final concentration was approx 1 g EDTA/L blood. For colonic mucosa, the colon and rectum were removed, flushed with 9 g/L NaCl, opened longitudinally, and the mucosa was scraped off with a microscope slide. Liver and mucosa were frozen in liquid nitrogen and stored at –70°C before analysis of DNA methylation, DNA methyltransferase activity, SAM and SAH.

## Genomic DNA Methylation

To assess the methylation status of CpG sites in genomic DNA, the in vitro methyl acceptance capacity of DNA was used *(6)* with minor modifications. This assay uses [$^3$H-methyl]SAM as a methyl donor and a prokaryotic CpG Dnmt1; the endogenous DNA methylation status is reciprocally related to the exogenous $^3$H-methyl incorporation. Briefly, DNA (2 µg) was incubated with 18.5 kBq of [$^3$H-methyl]SAM (Amersham Life Science, Piscataway, NJ), 60 m*M* SAM (New England Biolabs, Berverly, MA), 4 U of SssI methyltransferase (New England Biolabs), 1X NEBuffer 2 (New England Biolabs; 50 mmol NaCl/L, 10 mmol Tris-HCl/L, 10 mmol $MgCl_2$/L, and 1 mmol dithiothreitol/L, pH 7.9), in a final volume of 25 µL. All analyses were in duplicate. Genomic DNA for this assay was purified from tissue by using the DNeasy kit (Qiagen, Valencia, CA).

## DNA Methyltransferase (Dnmt) Activity

Tissue preparation: frozen liver (approx 100 mg) or scraped colonic mucosa (approx 50 mg) were homogenized with a Mark II Tissumizer (Tekmar, Cincinatti, OH) in 1 mL lysis buffer (per 1 L; 50 mmol Tris, pH 7.8, 1 mmol EDTA, 1 mmol dithiothreitol, 0.1 g sodium azide, 6 g phenylmethyl sulfolnyl fluoride, 100 mL glycerol, and 10 mL Tween 80) as previously described *(13).* This suspension was passed through an 18-gauge needle then through a 25-gauge needle. It was frozen at –70°C, and then thawed. The freeze–thaw cycle was repeated three times. The samples were stored at –70°C until analyzed. Protein concentrations were determined using the Bradford assay (Bio-Rad Laboratories, Richmond, CA).

Dnmt activity was determined by the procedure of Issa et al. *(13)* as modified by Davis and Uthus *(11).* Briefly, 10 µg of cellular protein were mixed with 0.5 µg of synthetic DNA consisting of repeats of inosine–cytosine [poly d(I-C).d(I-C), Amersham, Arlington Heights, IL] and 111 kBq Ci [$^3$H-methyl]SAM (80 Ci/mmol, Amersham, Arlington Heights, IL) in a total volume of 20 µL. This solution was incubated at 37°C for 2 h (colon) or 4 h (liver). The reaction was terminated by adding 350 µL of a stop solution (10 g sodium dodecyl sulfate/L, 2 mmol EDTA/L, 30 mg 4-aminosalicylate/L, 50 mL butanol/L, 125 mmol sodium chloride/L, 0.25 g carrier salmon testis DNA/L, and 1 g proteinase K/L). After an additional 30 min of incubation at 37°C, DNA was purified by phenol:chloroform extraction

and ethanol precipitation. RNA was removed by resuspension in 0.3 mol sodium hydroxide/L and incubation at 37°C for 45–60 min. The final solution was spotted onto Whatman GF/C filters. The filters were dried at 80°C, placed on a manifold, washed with 5 mL 50 g trichloroacetic acid/L containing 10 g bovine serum albumin/L, then washed with 3 mL of 700 mL ethanol/L, dried again at 80°C, placed in scintillation cocktail, and counted with a scintillation counter. Results are expressed as disintegrations per minute per 10 μg cellular protein per 4 h incubation (liver) or 2 h incubation (colon).

### *Liver and Colon S-Adenosylmethionine (SAM) and S-Adenosylhomocysteine (SAH)*

Portions of fresh liver and frozen colon were weighed and homogenized at 11,500 rpm in 0.4 mol $HClO_4$/L by using a Mark II Tissumizer (Tekmar, Cincinnati, OH). Samples were centrifuged at 2000*g* at 4°C for 30 min. Each supernatant was filtered through a 0.45 μm filter and stored at –70°C until analysis. SAM and SAH were measured on a Shimadzu LC-10 HPLC (Columbia, MD) equipped with a 250 × 4.6 mm Ultrasphere 5μ C18 IP column (Phenomenex, Torrance, CA) according to the procedure of Wagner et al. *(14).*

### *Liver and Colon Methionine Adenosyltransferase (MAT) Activity*

The activity of MAT was determined by the method of Cantoni *(15)* with modifications. No glutathione was included in the reaction mix, KCl was added to a final concentration of 0.3 *M,* and all volumes were reduced one-half resulting in a final volume of 0.5 mL. The reaction was stopped with 0.5 mL cold 0.4 *M* perchloric acid. After centrifugation, the supernatant fluid was diluted with $H_2O$ (50/50) and analyzed for SAM by capillary electrophoresis *(16).* Liver and colon were prepared by homogenization (1 g/4 mL) in 0.03 *M* potassium phosphate buffer, pH 7.0. The homogenate was centrifuged at 36,000*g* for 10 min at 4°C the supernatant was used for the assay. Protein concentrations were determined using the Bradford assay (Bio-Rad Laboratories, Richmond, CA).

### *Liver Glycine N-Methyltransferase (GNMT)*

The activity of liver GNMT was determined by the method of Cook and Wagner *(17).* Liver was homogenized in 5 volumes of buffer as described by Cook et al. *(18)* and centrifuged at 100,000*g* for 60 min at 4°C. The supernatant was diluted prior to analysis (1 volume supernatant fluid/4 volume 0.01 *M* potassium phosphate buffer, pH 7.4). Protein concentrations were determined using the Bradford assay (Bio-Rad Laboratories, Richmond, CA).

### Plasma Homocysteine and Glutathione

Total homocysteine and total glutathione were determined in heparinized plasma by using HPLC according to the procedure of Durand et al. *(19).*

### Selenium Status

Glutathione peroxidase (GPx) enzyme activity was determined by the coupled enzymatic method of Paglia and Valentine *(20),* which uses hydrogen peroxide (0.15 μ*M*) as the substrate. Thioredoxin reductase (TRR) activity was determined spectrophotometrically by the method of Holmgren and Bjornstedt *(21)* as modified by Hill et al. *(22).*

### Plasma Folate

Plasma folate was determined by RIA (KFSP, folate; Diagnostic Products, Los Angeles, CA).

### Statistical Analysis

The data were analyzed by a two-way ANOVA using the SAS general linear models program (SAS Version 8.02, SAS Institute, Cary, NC). Tukey's contrasts were used to differentiate among means for variables significantly ($p<0.05$) affected by an interaction between selenium and folate. Values are reported as means ± SEM.

## RESULTS

Dietary selenium did not affect body weight (297±3 vs 302±3 g, selenium-deficient vs adequate, respectively; data not shown). The selenium status indicators, liver GPx and TRR, were significantly reduced and plasma total glutathione was significantly elevated in rats fed the selenium-deficient diet (Table 1). Although plasma folate was significantly reduced (Table 1), dietary folate had no effect on weight gain (298±4 vs 301±3 g, folate-deficient vs adequate, respectively). Dietary selenium did not affect plasma folate and dietary folate did not affect indicators of selenium status (Table 1).

The interaction between dietary selenium and folate markedly affected ($p<0.0001$) the concentration of plasma total homocysteine (Fig. 1). Folate deficiency increased plasma homocysteine concentrations ($p<0.0001$). However, this increase was markedly attenuated in rats fed the selenium-deficient diet compared to those fed adequate selenium.

Folate deficiency decreased the concentration of SAM and increased the concentration of SAH, in the liver (Table 2). This resulted in a significant ($p<0.0001$) decrease in the SAM/SAH ratio. The increase of SAH as a result of folate deficiency was not as great in rats fed the selenium-defi-

Table 1
Effect of Dietary Selenium and Folate on Liver Glutathione Peroxidase (GPx) and Thioredoxin Reductase (TRR) Activities and the Concentration of Glutathione and Folate in Plasma[1]

| Diet | | Liver | Liver | Plasma | Plasma |
|---|---|---|---|---|---|
| Selenium | Folate | GPx | TRR | Glutathione | Folate |
| mg/kg diet | | mU/mg protein/min | | µmol/L | nmol/L |
| 0 | 0 | 7.8±1.9 | 3.6±0.8 | 15.7±1.45 | 9.54±1.37 |
| 0.2 | 0 | 1040±75 | 7.3±0.7 | 11.5±0.96 | 4.72±1.18 |
| 0 | 2 | 8.7±1.3 | 3.5±0.8 | 15.3±1.2 | 85.6±3.18 |
| 0.2 | 2 | 947±64 | 7.9±1.0 | 13.8±0.98 | 85.3±2.86 |
| ANOVA | | | | | |
| Selenium | | 0.0001 | 0.0001 | 0.03 | NS |
| Folate | | NS | NS | NS | 0.0001 |
| Selenium x Folate | | NS | NS | NS | NS |

[1] Values are means ± SEM, $n$ = 7–8 per group.
[2] NS = not significant, $p$>0.05.

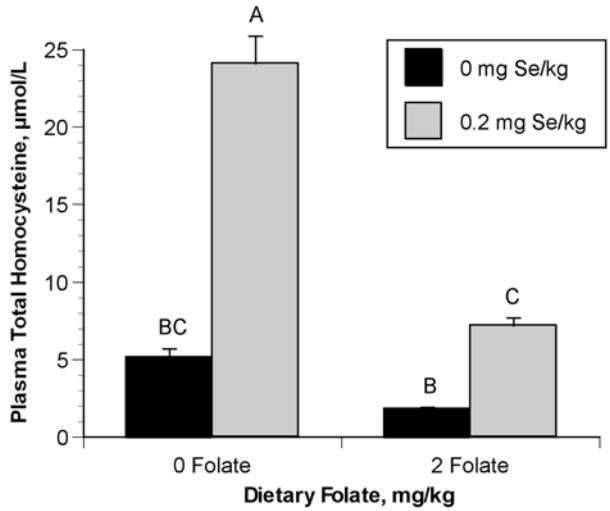


Fig. 1. Effect of dietary selenium and folate on plasma total homocysteine concentration in rats fed amino acid–based diets supplemented with 0 or 0.2 mg selenium/kg and 0 or 2 mg folate/kg diet in a 2 × 2 factorial design. Values are means ± SEM, $n$ = 8 per group. ANOVA; selenium, $p$<0.0001; folate, $p$<0.0001; selenium × folate, $p$<0.0001. Means without common letters differ, $p$<0.05.

Table 2
Effect of Dietary Selenium and Folate on Liver and Colon S-Adenosylmethionine (SAM), S-Adenosylhomocysteine (SAH), and SAM/SAH Ratio in Rats[1]

| Diet | | SAM | | SAH | | SAM/SAH | |
|---|---|---|---|---|---|---|---|
| Selenium | Folate | Liver | Colon | Liver | Colon | Liver | Colon |
| mg/kg diet | | nmol/g | | nmol/g | | | |
| 0 | 0 | 19.8±2.35 | 72.7±3.68 | 54.6±3.78$^{B}$ | 5.22±0.20$^{B}$ | 0.39±0.07 | 14.1±1.07 |
| 0.2 | 0 | 15.2±1.56 | 78.0±5.50 | 74.2±3.50$^{A}$ | 6.66±0.46$^{A}$ | 0.21±0.02 | 12.4±1.39 |
| 0 | 2 | 65.5±2.60 | 68.7±4.36 | 28.2±1.78$^{C}$ | 5.51±0.48$^{AB}$ | 2.38±0.13 | 13.2±1.52 |
| 0.2 | 2 | 67.1±4.08 | 74.7±3.31 | 31.3±1.25$^{C}$ | 4.60±0.28$^{B}$ | 2.17±0.16 | 16.8±1.74 |
| ANOVA | | | | | | | |
| Selenium | | NS[2] | NS | 0.0003 | NS | NS | NS |
| Folate | | 0.0001 | NS | 0.0001 | 0.03 | 0.0001 | 0.03 |
| Selenium x Folate | | NS | NS | 0.006 | 0.005 | NS | 0.005 |

[1] Values are means ± SEM, $n$ = 7–8 per group. Values in a column without a common letter differ, $p<0.05$.
[2] NS = not significant, $p>0.05$.

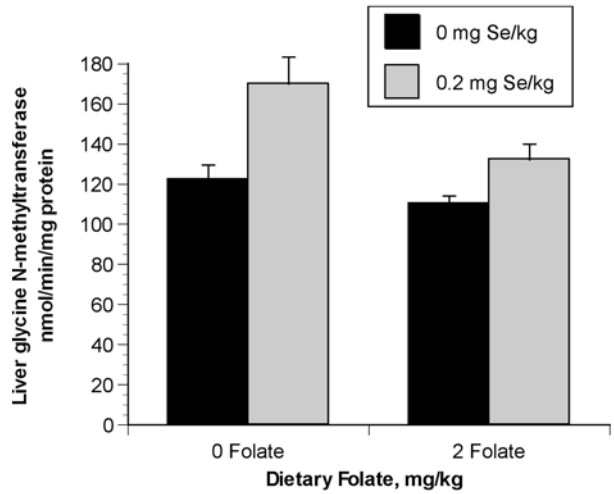


Fig. 2. Effect of dietary selenium and folate on activity of glycine N-methyltransferase from liver of rats fed amino acid–based diets supplemented with 0 or 0.2 mg selenium/kg and 0 or 2 mg folate/kg diet in a 2 × 2 factorial design. Values are means ± SEM, $n$ = 7–8 per group. ANOVA; selenium, $p<0.0003$; folate, $p<0.006$; selenium × folate, not significant.

cient diet compared to those fed adequate selenium. This resulted in a significant interaction between selenium and folate ($p<0.006$) on liver SAH.

Unlike liver SAM, colon SAM was not affected by dietary folate (Table 2). An interaction between selenium and folate affected colon SAH similar

Table 3
Effect of Dietary Selenium and Folate on DNA Methyltransferase Activity (Dnmt) and Global DNA Methylation in Liver and Colon of Rats[1]

| Diet | | Dnmt | | DNA $^3$H-methyl incorporation | |
|---|---|---|---|---|---|
| Selenium | Folate | Liver | Colon | Liver | Colon |
| mg/kg diet | | $^3$H Bq/10 μg protein | | $^3$H Bq/μg DNA | |
| 0 | 0 | 6.24±1.02 | 19.8±3.1 | 12.5±1.5 | 39.0±5.3 |
| 0.2 | 0 | 8.37±2.03 | 32.1±5.8 | 12.1±0.4 | 28.2±3.7 |
| 0 | 2 | 4.65±0.46 | 16.7±2.2 | 11.7±0.6 | 35.1±3.8 |
| 0.2 | 2 | 7.06±1.48 | 20.7±3.9 | 12.5±1.5 | 28.8±2.9 |
| ANOVA | | | | | |

[1] Values are means ± SEM, $n$ = 5–8 per group.
[2] NS = not significant, $p$>0.05.

to liver such that the highest concentration was found in rats fed a folate-deficient diet, adequate in selenium.

The activity of hepatic GNMT was increased by folate deficiency ($p$<0.006) and decreased by selenium deprivation ($p$<0.0003) (Fig. 2). Colon GNMT was not determined because sample was used up in other analyses.

Dnmt activity was not affected by selenium in liver but tended to be decreased ($p$<0.06) by selenium deficiency in the colon (Table 3). Global DNA methylation was (as indicated by greater incorporation of $^3$H-methyl) decreased ($p$<0.04) by selenium deficiency in colon but not liver. Dietary folate or its interaction with dietary selenium had no effect on liver or colon Dnmt or on liver or colon global DNA methylation.

## DISCUSSION

One-carbon metabolism is affected by both folate and selenium. This study confirms our earlier observation that showed dietary selenium and folate interacted to affect methyl metabolism in colon and liver of rats *(11).* It is well known that folate has a central role in one-carbon metabolism. However, the role of selenium in one-carbon metabolism has not been as extensively studied. In the early 1980s, work by Bunk and Combs *(23)* and Halpin and Baker *(24)* suggested that selenium deficiency can influence transsulfuration in chicks. Both groups also indicated that, quantitatively, free homocyst(e)ine was decreased in the plasma of selenium-deficient chicks. In a series of studies, Hill and Burk showed that in selenium deficiency liver glutathione production is increased and plasma

glutathione markedly elevated *(25–28).* Recently, our research showed that total plasma total homocysteine and plasma cysteine were significantly decreased by selenium deficiency in the presence of adequate folate *(29).* Furthermore, selenium, like folate, affects DNA methylation. In studies with cell culture and laboratory animals, we have shown that selenium deprivation results in DNA hypomethylation *(6,30).* Abnormal DNA methylation, including DNA hypomethylation, has been recognized as a constant molecular change in neoplasia *(31,32).*

When compared to selenium deprivation, the lack of response in either colon or liver DNA methylation to addition of dietary selenite (0.2 or 2.0 mg selenium/kg diet, current experiment and previous experiment, respectively) suggests that alterations in DNA methylation do not appear to be the mechanism for the chemopreventive effect of supranutritional concentrations of selenium given this model and time of exposure. However, these studies only determined the effect of dietary treatment on global DNA methylation. It is possible that gene-specific methylation could be affected. For example, we found that methylation of the p53 promoter region of Caco-2 cells was decreased as the selenium concentration of the culture media was decreased from 2 to 1 to 0 μmol/L *(6).*

Selenium compounds have been shown to inhibit Dnmt in cell culture models *(7).* However, these studies used purified Dnmt and very high concentrations of selenium. Our in vivo studies show that Dnmt activity in liver and colon is decreased by selenium deficiency when compared to rats fed supranutritional selenium *(11);* our current study shows a tendency for decreased colon Dnmt activity in selenium-deprived rats compared to rats fed adequate selenium. Whether these changes in Dnmt activity are related to increased tumorigenesis associated with selenium deficiency or the anticarcinogenic nature of supranutritional amounts of selenium is unknown at this time.

The current study also shows that methyl metabolism was markedly influenced by the interaction between dietary selenium and folate. As expected, plasma homocysteine was elevated in rats fed low folate. However, compared to rats fed 0.2 mg Se/kg this increase was attenuated by selenium deprivation. Therefore, the highest concentration of homocysteine was found in rats fed the low-folate diet with adequate selenium. Homocysteine is produced from enzymatic cleavage of SAH by SAH hydrolase *(33).* Because the equilibrium of this reaction favors the production of SAH from homocysteine *(33),* SAH will accumulate in situations where homocysteine accumulates (as in folate deficiency). This is of metabolic concern because methyltransferases that use SAM as the methyl donor are inhibited by the product SAH *(33).* Although tissue homocysteine was not measured, folate deficiency resulted in elevated liver SAH. Furthermore, similar to plasma homocysteine, the highest concentrations of SAH in liver and colon were found in the folate deficient rats fed 0.2 mg Se/kg.

Only liver SAM was decreased by folate deprivation. MAT, the enzyme that produces SAM from ATP and methionine, was not affected by

dietary folate or dietary selenium (data not shown). Therefore, the decrease in the concentration of liver SAM is most likely the result of decreased remethylation of homocysteine by the folate- dependent enzyme methionine synthase and/or altered use of SAM.

GNMT is an enzyme important in the regulation of tissue concentrations of SAM and SAH and hence SAM/SAH ratios *(17,34–37).* This regulation can take place through folate or by SAM itself. In times of low folate (e.g., folate deficiency) or excess SAM, the activity of the enzyme is increased *(34,37,38).* When SAM is low (e.g., low dietary methionine), GNMT is inhibited, thereby conserving SAM for important biological methylations. Regulation of GNMT has mainly been studied under conditions of altered methionine and/or folate intake *(34,37,38).* We measured GNMT activity because of the known effect of dietary folate on this enzyme and to ascertain whether changes in its activity could explain the effect of selenium and/or the interaction of selenium and folate, on methyl metabolism. Not only did we see the expected increased activity of GNMT with folate deficiency, but we also found that selenium deficiency decreases the activity in liver (Fig. 2). Because GNMT is a major enzyme in liver making up between 0.5% and 1% of the soluble protein in rat liver cytosol *(34),* it is likely that the changes seen in GNMT activity by dietary folate and selenium are of physiological significance. Furthermore, Yeo and Wagner *(39)* showed by immunohistochemical staining that GNMT is present in the jejunum. Future work will determine the effect of selenium and folate on mucosa GNMT. Presently we are unable to determine the significance of the changes in GNMT caused by dietary folate and selenium. However, the relevance may be reflected in tissue SAH where the concentrations of SAH are highest in liver and colon of rats fed the folate-deficient, selenium-adequate diet.

Although the concentration of SAH, an inhibitor of SAM-dependent methyltransferases, was highest in liver and colon from rats fed a folate-deficient, selenium-adequate diet, the activity of liver and colon Dnmt was unaffected by dietary treatment. Our previous study showed that both selenium deficiency and folate deprivation significantly decreased liver and colon Dnmt activity. Our current study showed similar patterns with dietary selenium but did not reach statistical significance. Dietary selenium only affected colon global DNA methylation; DNA from colon was hypomethylated (as indicated by the greater incorporation of $^{3}$H-methyl) in rats fed a selenium-deficient diet. Thus, although SAH was elevated in colon and liver of rats fed a folate-deficient, selenium-adequate diet, Dnmt activity and global DNA methylation were unaffected in this dietary group. These differences between colon and liver are most likely not the result of differences of selenium status because we have shown that selenium concentrations in colon parallel those in liver *(30,40).*

All of the results found in this study in which the concentration of selenium supplemented to the diet was 0.2 mg/kg were similar to the results in our previous study in which the concentration of selenium

supplemented to the diet was 2 mg/kg. This suggests that selenium deficiency appears to be a more important modifier of methyl metabolism than either adequate or supplemental selenium.

This, however, does not rule out the importance of these findings in determining the mechanisms of how selenium may impact carcinogenesis. For example, Lu and Jaing *(4)* stated: "The physiochemistry of selenium delivery to transformed epithelial cells in in vivo lesions may be a major determinant of the actual mechanism(s) as well as the processes that are invoked to regulate growth and fate of the solid lesion." They speculated that selenium delivery to the interior avascular lesions may be reduced resulting in a conditional selenium deficiency within tumor. To this end, their model predicts that more selenium is required to enrich selenium metabolic pools within the avascular lesions in order to elicit the chemopreventive mechanisms of selenium. Without enrichment of the pool, one-carbon metabolism, for example, within the tumor would then follow metabolism similar to that seen in selenium deficiency. This ultimately could contribute to progression of tumorigenesis. Additionally, it is known that GNMT expression and activity are decreased in tumor tissue *(41,42).* Functional characterization of GNMT showed that GNMT is able to bind benzo[*a*]pyrene (BaP) and decrease BaP–DNA adduct formation *(43,44).* Therefore, GNMT can be classified as a tumor susceptibility gene; this enzyme is decreased by selenium deprivation.

In summary, our results show that dietary selenium and folate interact in affecting one-carbon metabolism. Additionally, we found that GNMT activity is differentially affected by dietary folate and selenium; folate deficiency increases, whereas selenium deprivation decreases, the activity of GNMT. We also show that the effects of selenium on one-carbon metabolism are likely the result of selenium deprivation. This suggests that the antitumorigenic effects of selenium that are associated with supranutritional concentrations likely follow a different mechanism. However, as possibly seen in the interior of avascular lesions, conditional selenium deficiency in the presence of adequate dietary selenium, may have a role in the progression of tumorigenesis through effects on one-carbon metabolism.

## ACKNOWLEDGMENTS

The authors thank Denice Schafer and her staff for care of the animals, Sheila Bichler and LuAnn Johnson for performing statistical analysis, and Kim Baurichter and Thomas Zimmerman for technical assistance.

## REFERENCES

1. C. D. Davis and J. W. Finley, in *Functional Foods and Nutraceuticals in Cancer Prevention,* R. R. Watson, ed. Iowa State Press, Ames, 2003, pp. 55–85.

2. H. E. Ganther, Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase, *Carcinogenesis* **20,** 1657–1666 (1999).
3. C. Ip, Y. Dong, and H. E. Ganther, New concepts in selenium chemoprevention, *Cancer Metastasis Rev.* **21,** 281–289 (2002).
4. J. Lu and C. Jiang, Antiangiogenic activity of selenium in cancer chemoprevention: metabolite-specific effects, *Nutr. Cancer* **40,** 64–73 (2001).
5. G. F. Combs, Jr., Chemopreventive mechanisms of selenium, *Med Klin* **94** (Suppl 3), 18–24 (1999).
6. C. D. Davis, E. O. Uthus, and J. W. Finley, Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon, *J. Nutr.* **130,** 2903–2909 (2000).
7. E. S. Fiala, M. E. Staretz, G. A. Pandya, K. El-Bayoumy, and S. R. Hamilton, Inhibition of DNA cytosine methyltransferase by chemopreventive selenium compounds, determined by an improved assay for DNA cytosine methyltransferase and DNA cytosine methylation, *Carcinogenesis* **19,** 597–604 (1998).
8. Y. I. Kim, Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies, *Environ. Mol. Mutagen.* **44,** 10–25 (2004).
9. S. W. Choi and J. B. Mason, Folate and carcinogenesis: an integrated scheme, *J. Nutr.* **130,** 129–132 (2000).
10. C. La Vecchia, E. Negri, C. Pelucchi, and S. Franceschi, Dietary folate and colorectal cancer, *Int. J. Cancer* **102,** 545–547 (2002).
11. C. D. Davis and E. O. Uthus, Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one-carbon metabolism in rats, *J. Nutr.* **133,** 2907–2914 (2003).
12. P. G. Reeves, Components of the AIN-93 diets as improvements in the AIN-76A diet, *J. Nutr.* **127,** 838S–841S (1997).
13. J. P. Issa, P. M. Vertino, J. Wu, et al., Increased cytosine DNA-methyltransferase activity during colon cancer progression, *J. Natl. Cancer Inst.* **85,** 1235–1240 (1993).
14. J. Wagner, N. Claverie, and C. Danzin, A rapid high-performance liquid chromatographic procedure for the simultaneous determination of methionine, ethionine, S-adenosylmethionine, S-adenosylethionine, and the natural polyamines in rat tissues, *Anal. Biochem.* **140,** 108–116 (1984).
15. G. L. Cantoni, in *Methods in Enzymology,* Vol. II, S. P. Colowick and N. O. Kaplan, eds., Academic Press, New York, 1955, pp. 254–256.
16. E. O. Uthus, Simultaneous detection of S-adenosylmethionine and S-adenosylhomocysteine in mouse and rat tissues by capillary electrophoresis, *Electrophoresis* **24,** 1221–1226 (2003).
17. R. J. Cook and C. Wagner, Glycine N-methyltransferase is a folate binding protein of rat liver cytosol, *Proc Natl Acad Sci USA* **81,** 3631–3634 (1984).
18. R. J. Cook, D. W. Horne, and C. Wagner, Effect of dietary methylgroup deficiency on one-carbon metabolism in rats, *J. Nutr.* **119,** 612–617 (1989).
19. P. Durand, L. J. Fortin, S. Lussier-Cacan, J. Davignon, and D. Blache, Hyperhomocysteinemia induced by folic acid deficiency and methionine load—applications of a modified HPLC method, *Clin. Chim. Acta* **252,** 83–93 (1996).
20. D. Paglia and W. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.* **70,** 158–169 (1967).
21. A. Holmgren and M. Bjornstedt, Thioredoxin and thioredoxin reductase, *Methods Enzymol.* **252,** 199–208 (1995).
22. K. E. Hill, G. W. McCollum, and R. F. Burk, Determination of thioredoxin reductase activity in rat liver supernatant, *Anal. Biochem.* **253,** 123–125 (1997).
23. M. J. Bunk and G. F. Combs, Jr., Evidence for an impairment in the conversion of methionine to cysteine in selenium-deficient chick, *Proc. Soc. Exp. Biol. Med.* **167,** 87–93 (1981).

24. K. M. Halpin and D. H. Baker, Selenium deficiency and transsulfuration in the chick, *J. Nutr.* **114,** 606–612 (1984).
25. K. E. Hill and R. F. Burk, Effect of selenium deficiency and vitamin E deficiency on glutathione metabolism in isolated rat hepatocytes, *J. Biol. Chem.* **257,** 10668–10672 (1982).
26. K. E. Hill and R. F. Burk, Effect of selenium deficiency on the disposition of plasma glutathione, *Arch. Biochem. Biophys.* **240,** 166–171 (1985).
27. K. E. Hill, R. F. Burk, and J. M. Lane, Effect of selenium depletion and repletion on plasma glutathione and glutathione-dependent enzymes in the rat, *J. Nutr.* **117,** 99–104 (1987).
28. K. E. Hill, M. A. Taylor, and R. F. Burk, Influence of selenium deficiency on glutathione disulfide metabolism in isolated perfused rat heart, *Biochim. Biophys. Acta.* **923,** 431–435 (1987).
29. E. O. Uthus, K. Yokoi, and C. D. Davis, Selenium deficiency in Fisher-344 rats decreases plasma and tissue homocysteine concentrations and alters plasma homocysteine and cysteine redox status, *J. Nutr.* **132,** 1122–1128 (2002).
30. C. D. Davis and E. O. Uthus, Dietary selenium and azadeoxycytidine treatment affect dimethylhydrazine-induced aberrant crypt formation in rat colon and DNA methylation in HT-29 cells, *J. Nutr.* **132,** 292–297 (2002).
31. B. Richardson and R. Yung, Role of DNA methylation in the regulation of cell function, *J. Lab. Clin. Med.* **134,** 333–340 (1999).
32. C. D. Davis and E. O. Uthus, DNA methylation, cancer susceptibility, and nutrient interactions, *Exp. Biol. Med. (Maywood)* **229,** 988–995 (2004).
33. J. D. Finkelstein, Pathways and regulation of homocysteine metabolism in mammals, *Sem. Thromb. Hemost.* **26,** 219–225 (2000).
34. C. Wagner, W. T. Briggs, and R. J. Cook, Inhibition of glycine N-methyltransferase activity by folate derivatives: implications for regulation of methyl group metabolism, *Biochem. Biophys. Res. Common.* **127,** 746–752 (1985).
35. F. M. Loehrer, W. E. Haefeli, C. P. Angst, G. Browne, G. Frick, and B. Fowler, Effect of methionine loading on 5-methyltetrahydrofolate, S-adenosylmethionine and S-adenosylhomocysteine in plasma of healthy humans, *Clin. Sci. (Colch.)* **91,** 79–86 (1996).
36. K. Aida, M. Tawata, M. Negishi, and T. Onaya, Mouse glycine N-methyltransferase is sexually dimorphic and regulated by growth hormone, *Horm. Metab. Res.* **29,** 646–649 (1997).
37. H. Ogawa, T. Gomi, F. Takusagawa, and M. Fujioka, Structure, function and physiological role of glycine N-methyltransferase, *Int. J. Biochem. Cell. Biol.* **30,** 13–26 (1998).
38. M. Balaghi, D. W. Horne, and C. Wagner, Hepatic one-carbon metabolism in early folate deficiency in rats, *Biochem. J.* **291** (Pt 1), 145–149 (1993).
39. E. J. Yeo and C. Wagner, Tissue distribution of glycine N-methyltransferase, a major folate-binding protein of liver, *Proc. Natl. Acad. Sci. USA* **91,** 210–214 (1994).
40. J. W. Finley, C. D. Davis, and Y. Feng, Selenium from high selenium broccoli protects rats from colon cancer, J Nutr 130, 2384–9 (2000).
41. H. H. Liu, K. H. Chen, Y. P. Shih, W. Y. Lui, F. H. Wong, and Y. M. Chen, Characterization of reduced expression of glycine N-methyltransferase in cancerous hepatic tissues using two newly developed monoclonal antibodies, *J. Biomed. Sci.* **10,** 87–97 (2003).
42. J. E. Heady and S. J. Kerr, Alteration of glycine N-methyltransferase activity in fetal, adult, and tumor tissues, *Cancer Res.* **35,** 640–643 (1975).
43. R. Bhat, C. Wagner, and E. Bresnick, The homodimeric form of glycine N-methyltransferase acts as a polycyclic aromatic hydrocarbon-binding receptor, *Biochemistry* **36,** 9906–9910 (1997).
44. S. Y. Chen, J. R. Lin, R. Darbha, P. Lin, T. Y. Liu, and Y. M. Chen, Glycine N-methyltransferase tumor susceptibility gene in the benzo(a)pyrene-detoxification pathway, *Cancer Res.* **64,** 3617–3623 (2004).